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BLUE LIGHT-INDUCED REACTIVITY OF RETINAL AGE PIGMENT: IN VITRO GENERATION OF OXYGEN REACTIVITY.

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Purpose. To determine whether the retinal pigment epithelium (RPE) exhibits any substantial phototoxicity and, if so, which are the major chromophores involved.
Methods. Intact human RPE cells were isolated from the posterior poles of human and bovine eyes. Lipofuscin and melanosome granules were also isolated from human donor eyes. Action spectra of oxygen photo-consumption were determined by ESR oximetry and hydrogen peroxide photo-formation using an oxidase electrode. The spectral range for blue light effects was 408-495nm at a fluence rate of ~220 mW/cm^2. Superoxide anions were determined using a DMPO spin probe and singlet oxygen determined using cholesterol as an acceptor. Lipid peroxidation was assessed by an iodometric assay.
Results. Illumination of human RPE cells induced a significant uptake of oxygen that was both wavelength and age-dependent. Analysis of photoreactivity of RPE cells and their age pigments, lipofuscin, indicated that the photoreactivty of RPE cells is primarily due to the presence of lipofuscin which generates several oxygen reactive species including singlet oxygen, superoxide anion and hydrogen peroxide. It was also observed that lipofuscin photosensitized aerobic reactions led to enhanced lipid peroxidation as measured by accumulation of lipid peroxides and malondialdehyde in illuminated pigment granules.
Conclusions. It is postulated that lipofuscin is a potential photosensitizer and may increase the risk of retinal phøtodamage and ARMD.

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THE DOPAMINERGIC CELLS OF THE RAT RETINA FOLLOWING PRENATAL EXPOSURE TO COCAINE. AN IMMUNOCYTOCHEMICAL AND NEUROCHEMICAL STUDY

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***Purpose*: The purpose of this study was to assess the long-term effects of prenatal exposure to cocaine on the dopaminergic cells of the retina of the rat, following prenatal exposure to cocaine.**
***Methods*: Pregnant Wistar rats were given 60 mg/Kg body weight/day of cocaine hydrochloride (SIGMA), s.c., from gestational days 8-22. At postnatal days (PND) 14, 30 and 90 the male offspring were transcardially perfused with fixative, the retina processed as whole-mount preparations and immunostained with the antibody anti-tyrosine hydroxylase (TH). Rats from other groups were decapitated at the same postnatal ages and processed by HPLC for determination of the levels of dopamine and its metabolites (DOPAC, HVA).**
***Results*: The number and density of large-TH cells did not differ significantly between prenatally exposed retinas and respective controls at any age (PND 14, 30 and 90). The density of the small TH-cells was significantly reduced in the temporal, nasal, superior and inferior hemiretinas of PND14 cocaine exposed retinas when compared with age-matched controls. The neurochemical data showed differences at PND14 between the cocaine group and the controls .**
***Conclusions*: Prenatal cocaine exposure produced changes in the developing dopaminergic system of the rat retina, both in the population of the small TH-cells and the levels of the dopamine and its metabolites.**
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GABA$_A$ RECEPTOR SUBUNITS IN IDENTIFIED AMACRINE CELL TYPES IN THE RABBIT RETINA

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Purpose: The circuitry of the movement detection systems of the retina is not well known, although GABA$_A$ receptors and starburst amacrine cells are very likely involved. Therefore, we have analyzed the distribution of GABA$_A$ receptor subunit proteins in identified neurons in the rabbit retina, including starburst amacrine cells.
Methods: Rabbit retina whole mounts were stained with selective monoclonal GABA$_A$ receptor subunit antibodies and examined in a confocal microscope. Starburst amacrine cells and other cells in the inner retina stained by intraocular injection of DAPI were classified by intracellular injections of Lucifer yellow in living tissue. Starburst amacrine cells were also identified with choline acetyltransferase immunohistochemistry.
Results: No or only very little GABA$_A$ receptors (subunits α1 and β1/β2) were found in starburst amacrine cells. On the other hand, such receptors occur in high concentration in a cell type with morphologically characteristic features, called the DAPI-3 cell and belonging to the ones stained by an intraocular DAPI injection. This can be used as an aid for finding it for intracellular dye injections. Its perikarya are located in the proximal cell rows of the inner nuclear layer and their processes ramify in two sublayers in the inner plexiform layer, where they abut the processes of the two mirror symmetric populations of starburst amacrine cells. They occur with roughly the same density as OFF starburst amacrine cells. GABA$_A$ receptor proteins also appear in other retinal cell types at lower concentrations.
Conclusions: The high GABA$_A$ receptor content of the DAPI-3 cells can be used for identifying them immunohistochemically. Movement detection is likely to involve complex circuits formed by several different cells, including the ones studied here. Different GABA$_A$ receptor subtypes appear to play different roles.

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GUANINE NUCLEOTIDE (GN) EXCHANGE NOISE AND ROD G PROTEIN STABILITY

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Purpose: To determine rate and thermal dependence of GDP/GTP exchange of rod G protein under various conditions. Method: α^{32}P GTP or GTPγ^{35}S was incubated with G_α•GDP or $G_{\alpha\beta\gamma}$•GDP at temperatures from 10 to 37° C. and nitrocellulose filtered at various times to determine rates of GN exchange. G protein deterioration was controlled for by determining maximum exchange catalyzed by stripped rod membranes in the light at comparable times. Results: Rates varied from hours to days with a Q_{10} of 5 or more. Arrhenius slopes gave activation enthalpies of 25-30 kcal mole^{-1} for both exchange and decay (loss of ability to exchange) suggesting a common activation step for both processes. Decay was faster in absence of ambient nucleotide but had the same Arrhenius slope. Conclusion: Rod G protein can activate by spontaneous nucleotide exchange in the absence of bleached rhodopsin. The rate of activation at body temperature is about 10^3 s^{-1} rod^{-1}. Coupled to rod PDE, it can explain the measured dark rate of cGMP turnover. This rate is dramatically augmented in the presence of trace contamination by disk membranes, even after hydroxylamine treatment, consistent with the Dowling-Rushton effect of light adaptation by unregenerated visual pigment and with more recent electrophysiological results of C. Cornwall et. al. (1994/1995). The very similar Arrhenius slopes for seemingly diverse aspects of G protein activity suggests that both loss of activity and nucleotide binding reflect a common unfolding step in the protein structural dynamics. Supported by U.S.P.H.S. National Eye Inst. EY00012 and EY01583.